

REMARKS

The Specification has been amended to include SEQ ID numbers which were omitted at the time of filing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made.".

The undersigned hereby states that the paper copy Sequence Listing and the computer readable form copy (CRF copy) of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.825(a) and (b), respectively, are the same and contain no new matter. Accordingly, entry of the Sequence Listing into the above-captioned case is respectfully requested.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 220002063600. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Paragraph [0014] at page 4 has been amended as follows:

[0014] Figure 1A shows diagrammatically plasmids containing the bicistronic system of the invention. Figure 1B-1 - 1B-6 shows the flow cytometric (FACS) analysis of HEK293 cells transfected with pDF-E and pDF-e. Dot plots represent blue fluorescence (Y-axis) vs. green fluorescence (X-axis). Approximately 30% of the cells were transfected in this experiment. Region MI in the histogram plots represents transfected cells, and the mean fluorescence value of these cells is shown above each histogram.

Paragraph [0015] at page 4 has been amended as follows:

[0015] Figure 2A shows the strategy for screening for IRES elements. The input plasmid library containing two fluorescent reporters is introduced into eukaryotic cells by protoplast fusion. Small shaded circles represent *E. coli*, and large shaded circles represent HEK293 cells. A suitable sorting window is used to isolate double-positive cells via FACS, and plasmids are extracted by alkaline lysis and electroporated into bacteria. Figure 2B-1 - 2B-3 shows a pilot experiment to establish screening conditions. The input pool, whose collective sequence is shown in the left panel, contained a mixture of plasmids in the ratio of 10000 pDF-e:1 pDF-E. After three rounds of screening for strong (pDF-E) IRES elements, 700 positively selected clones were obtained; their collective sequence is shown in the right panel. In the middle panel is the collective sequence obtained when the sorting window was positioned so as to select poor (pDF-e) IRES elements. IRES positions 299-302, which have been mutated to create pDF-e, are shown in boldface; the sequence of pDF-e at these positions is 5'-AAAG-3', while the sequence

of pDF-E is 5'-GCGA-3'. Figure 2C shows the formulas of five nucleotide sequences identified by the method of the invention as potential IRES elements (SEQ ID NOS: 1-5).

Paragraph [0016] at page 5 has been amended as follows:

[0016] Figure 3A - 3D shows flow cytometric analysis of clones recovered from screen. Protoplasts were made from selected plasmids and fused to HEK293 cells. After 24 hours, cells were analyzed via flow cytometry for blue (Y-axis) and green (X-axis) fluorescence. PS2, PS3, and PS4 are positively selected clones, while NS I is a randomized 50-nt element that does not possess significant IRES activity. As a negative control, protoplasts from a control plasmid (pLuc-NI) that does not express fluorescent proteins was fused to HEK293 cells. Approximately 10% of HEK293 cells were productively fused with protoplasts.

Paragraph [0018] at page 6 has been amended as follows:

[0018] Figure 5A - 5C shows IRES elements PS3 and PS4 compete with PV IRES-mediated translation *in vitro*.

Paragraph [0019] at page 6 has been amended as follows:

[0019] Figure 6A - 6B shows proposed secondary structures of PS3 and PS4 with an enzymatic digestion map. Triangles represent nucleotides reactive to RNase T1 and circles mark nucleotides reactive to nuclease S1. Solid symbols represent strong reactivities, while open symbols represent weak reactivities. RNase VI, which we have previously used to identify basepaired regions of other RNA's, is not currently available to the scientific community and hence was not used in this study.

Paragraph [0035] at page 10 has been amended as follows:

[0035] To create a randomized library for inclusion in pDF-lib, a PAGE-purified 50 nucleotide-long randomized oligonucleotide library (IDT) with sequence 5'-GCGCACTGATGAATTN₅₀-GGATCCTCAGACTCCG-3' (SEQ ID NO: 6) was obtained. The phosphoramidite ratio for random sequence DNA synthesis was normalized to account for

differing coupling rates (Unrau, P. J., *et al.*, *Nature* (1998) 395:260-263). The oligonucleotide pool was amplified by 10 cycles of PCR as described by Tuerk, C., *Methods in Mol. Biol.* (1997) 67:219-230, and the amplified DNA was cut with *Eco*RI and *Bam*HI and ligated to *Eco*RI-*Bam*HI-digested pDF-N in ten separate reactions. Each ligation reaction was divided into two parts, and electroporated into DH5 α cells. Each transformation yielded approximately 50,000 colonies; colonies were combined to yield a total pool of approximately 1×10^6 transformants.

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By:

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